



TEXAS A&M  
UNIVERSITY

TEXAS A&M COLLEGE OF  
MEDICINE

# FLOW CYTOMETRY AND CELL SORTING FACILITY

WHAT YOU NEED TO KNOW TO GET  
STARTED



# SIGN UP FOR AN iLAB ACCOUNT

To gain access to the FCCSF scheduling calendars, you must register for an iLab account:

1. Navigate to the core page: In the upper-right-hand corner of the screen where it says, 'Welcome TAMU user, please click here to login or register,' select the *click here* [FCCSF iLab page](#)
2. You will be directed to an authentication page where you will need to enter your TAMU credentials
3. Once you have entered your credentials, click the 'Login' button
4. You will be directed to an iLab Registration page where you will need to select your PI/Lab, and verify your contact information
5. **Once your registration has been submitted, your PI will receive a notification that you have requested membership to their lab in iLab. They will need to approve your membership and assign any TAMU Fund Name for your use.**
6. Let me know when you have registered, and I will grant you access to our calendars.
7. For assistance with any iLab related issues contact TAMU Core Coordinator, Ashlyn Montgomery, at [amontgomery@tamu.edu](mailto:amontgomery@tamu.edu)

# RATES

MACS Quant Tyto Sorter - \$55/ hour plus cartridge cost

Service	TAMU	External
Aria Analysis	\$40/hr	\$60/hr
Aria Analysis w/ Operator	\$55/hr	\$83/hr
Aria Sort	\$55/hr + 1.5 hr for setup	\$83/hr + 1.5 hr for setup
Aria Sort w/ Operator	\$70/hr + 1.5 hr for setup	\$105/hr + 1.5 hr for setup
Seahorse	\$10/hr	\$15/hr
Fortessa	\$40/hr	\$60/hr
Fortessa w/ Operator	\$50/hr	\$75/hr
Cytek Analysis	\$40/hr	\$60/hr
Cytek Analysis w/ Operator	\$50/hr	\$75/hr
Training on all equipment	No charge	No charge

# SCHEDULING AND CANCELLATION

- Users for the Aria, Cytek, and Fortessa must reserve at least 30 minutes, to ensure enough time to run samples and clean the instrument when finished.
- Individuals wishing to reserve more than 5 contiguous hours of time on the Aria, Cytek, and Fortessa must submit a request in writing to the FCCSF explaining the need for >5 hours of contiguous time.
- Reservations may be cancelled >12 hours ahead of scheduled time without charge.
- Reservations cancelled less than 12 hours prior to scheduled time will be charged 100% of the total reservation time.
- No shows will be charged 100% of the total reservation time.

# BILLING

- Billed time for instruments begins at the start of the reserved time. Billed time for instruments ends when the user clicks “Finish” in iLab kiosk.
- If a user goes over the reserved time, which is only allowed if no one is scheduled immediately after the reserved time, billed time will begin at the beginning of the scheduled time until the time that the user clicks “Finish” in iLab kiosk, even if it extends beyond the reserved time.
- For all sorting reservations, 1.5 hours will be added as a sorter setup charge.

# BIOSAFETY-RISK GROUP 2

- Our lab is BSL2 and you may run BSL2 agents on our instruments, provided you follow BSL2 biosafety precautions and are current with Texas A&M Biosafety and EHS trainings.
- If your agent falls into the category of Risk Group 2, i.e. human cells, infected cells or genetically modified cells, please add our facility building and room to your IBC protocol and state in protocol which agents you will be using in our facility and on which instruments.
- Provide biosafety information on your RG2 agents

# BUILDING AND LAB ACCESS FOR NON-HSC LABS

- We are located at the HSC Campus in MREB2 Room 3226
- Building door and lab hallway require badge access
- Non-HSC customers should enter the building through the loading dock and notify Security that you need to check out a FCCSF badge for the day.
- If you would like an HSC badge, let staff know, and they will send you the appropriate paperwork.

# FCCSF INSTRUMENTS

- BD Fortessa X-20 – 4 Laser, 16-color Flow Cytometry Analyzer
- BD FACSAria II Cell Sorter - 4 Laser, 13-color droplet based Cell Sorter
- Miltenyi MACSQuant Tyto 3 Laser, 8-color, Cartridge-Based Cell Sorter
- Cytex Aurora Full Spectrum Flow Cytometry Analyzer- 5 lasers and 64 detectors
- Curiox HT2000 Laminar Flow Cell Washer
- Vi-Cell Cell Counter and Viability Analyzer
- Miltenyi Octo-Dissociator – tissue dissociator for use with Miltenyi kits
- Seahorse XFe96 Metabolic Analyzer



# CURIOX LAMINAR FLOW CELL WASHER



## Advantages

- Standardized procedure
- Less stressful on cells
- Saves time
- Saves antibody
- Greater cell retention
- Decrease sample debris

## Get Started

- Principles of Operation: [Curiox: How it Works](#)
- Watch training video: [FCCSF Curiox Tutorial Video](#)
- Schedule training on iLab calendar
- Purchase plate and buffer cap
- Titrate antibodies using the Curiox
- Contact Curiox Application Scientist to optimize staining protocol: [MarkA@curiox.com](mailto:MarkA@curiox.com)

# Vi-CellXR Cell Viability Analyzer

## Advantages

- Automated trypan blue exclusion method
- Takes up to 100 images per run
- Faster and more accurate than hemocytometer
- No charge for use
- Consumables provided
- Customizable parameters



## Getting Started

- No iLab scheduling required
- Watch training video: [Vi-Cell Operation](#)
- System specs
  - Cell Diameter Range 30-70 $\mu$ m
  - Cell Concentration Range  $5 \times 10^4$  –  $1 \times 10^7$
  - Sample Volume .5ml-2.0ml
  - Counting Accuracy  $\pm 6\%$
  - Acquisition time – 2 minutes

# MILTENYI gentleMACS OCTO DISSOCIATOR WITH HEATERS

## Advantages

- Standardized and optimized tissue dissociation
- Two units with 8 slots each
- No charge for use
- Pre-defined and custom protocols



## Get Started

- How it works – [Miltenyi OctoDissociator](#)
- Training video – [FCCSF Dissociator Tutorial](#)
- Contact Technical Application Scientist to determine the best kit for your application
- Use requires an iLab reservation. Please schedule use on iLab calendar

# 16 Fluorescent Detectors

## 4 Lasers

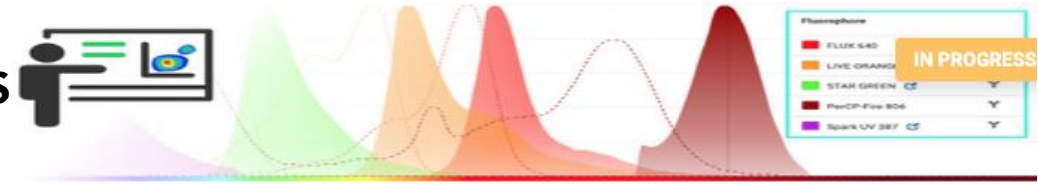
- 405- Violet
- 488 – Blue
- 561 – Yellow green
- 640 -Red

	LASER (NM)	BP	LP	OPTIMAL COLORS			Additional optimal fluorochrome options available on FluoroFinder.com.		
LASER	405	450 / 50	--	Brilliant Violet 421	Alexa Fluor 405	CF405M	DyLight 405	eFluor 450	
				Pacific Blue	SuperBright 438	V450	VioBlue		
		525 / 50	505	Brilliant Violet 510	Alexa Fluor 430	DyLight 405LS	V500	VioGreen	
				Brilliant Violet 605	DyLight 405LS	eFluor 605NC	eVolve 605	Qdot 605	
		610 / 20	600	SuperBright 600					
				Brilliant Violet 650	eFluor 650NC	eVolve 655	Qdot 655	SuperBright 645	
670 / 30	635	Brilliant Violet 711	Qdot 705	SuperBright 702					
710 / 50	685	Brilliant Violet 785	Qdot 800						
LASER	488	525 / 50	505	FITC	Alexa Fluor 488	BB515	Cy2	DyLight 488	
				Fluorescein	VioBright FITC				
LASER	561	695 / 40	655	PerCP-Cy5.5	BB700	PerCP-eFluor 710	PerCP-Vio700		
				PE					
		610 / 20	600	PE-Texas Red	DyLight 594	ECD	PE-CF594	PE-Dazzle 594	
				PE-eFluor 610	PE-Vio615				
		670 / 30	635	PE-Cy5					
				PE-Cy5.5					
780 / 60	750	PE-Cy7	PE-Vio770						
LASER	640	670 / 30	--	APC	Alexa Fluor 647	Cy5	DyLight 633	NL 637	
				APC-Alexa 700					
		730 / 45	685	APC-Cy7	APC-Alexa 750	APC-eFluor 780	APC-Fire 750	APC-H7	
APC-Vio770									

# FLUOROFINDER TRAINING

1. Sign up for an account at <https://fluorofinder.com/> using your @tamu.edu email. This will enable you to access all the premium features of FluoroFinder, including training.
2. Log in and navigate to Flow Cytometry Training.
3. Complete Flow Cytometry Essentials before coming to your first hands-on training.
4. If you are using the Cytex Aurora, also complete Spectral Flow.
5. If you are sorting on the Aria, complete the sorting module.

## FLOW BASICS



### Flow Cytometry Essentials

Covering common concepts from principles and instrumentation to panel design, measurement, analysis, and statistics

## SPECTRAL FLOW



### Cytometry Select 2

Acquire the skills to design, perform, and analyze any spectral flow cytometry experiment

## SORTING



### Cytometry Select 1

Gain a comprehensive understanding of advanced cell sorting topics from instrument optimization to single-cell deposition

# FORTESSA HANDS-ON TRAINING

- Review PPT: [Setting up an experiment in Diva on the Fortessa](#)
- Watch Video: [BD Fortessa X-20 - Setting up an experiment in Diva](#)
- Confirm staff availability and schedule training session in iLab
  - [Setting up an Experiment in Diva using HTS](#)

# CYTEK AURORA 5-LASER FULL SPECTRUM FLOW CYTOMETRY ANALYZER

- Spectral Cytometry Theory
  - [OpenFlow: Full Spectrum Flow Cytometry with the Cytex Aurora](#)
  - Fluorofinder Spectral Flow Cytometry video training series
- Hands-on Training- review basic materials, confirm staff availability and schedule in iLab
  - [SpectroFlo Tutorials](#) – Watch “Create and Unmix and Experiment”, and SpectroFlo Overview
  - [Cytex Webinar: Applying Panel Design Principles -Dr. Maria Jaimes](#)
  - [Cytex Webinar: Best Practices for Reference Controls Optimization by Dr. Maria Jaimes](#)

# OTHER CYTEK RESOURCES AND NOTES

- University of Chicago Aurora Training materials – A lot of useful information here
  - [Spectral Flow Cytometry](#)
  - [Spectral Experiment Design](#)
  - [UChicago Aurora Training Course](#)
- Notes:
  - [How to Effectively Evaluate Data Quality – Video](#)
  - [How to Evaluate Data Quality – PDF](#)
  - Sign up for Cytel Cloud account [Cytel Cloud Login](#)
    - This allows you to use their spectral viewer and set up SpectroFlow experiment online
  - When designing a new panel for the Aurora, it is critical to take the time needed to optimize fluorophore choice, properly titrate your antibodies and find optimal reference controls for unmixing.



# BD FACSAria II CELL SORTER

## 13 Fluorescent Detectors

### Capabilities-Lasers and filters

#### 4 Lasers

- 405- Violet
- 488 – Blue
- 561 – Yellow green
- 640 -Red

	LASER (NM)	BP	LP	OPTIMAL COLORS					Additional optimal fluorochrome options available on FluoroFinder.com.
LASER	405	450 / 50	--	Alexa Fluor 405 DyLight 405	Brilliant Violet 421 eFluor 450	Cascade Blue SuperBright 436	Pacific Blue V450	CF405M VioBlue	
		525 / 50	--	AmCyan V500	Brilliant Violet 510 VioGreen	Cascade Yellow	Alexa Fluor 430	DyLight 405LS	
		660 / 40	--	Brilliant Violet 650	eFluor 650NC	Qdot 655	eVolve 655	SuperBright 645	
LASER	488	530 / 30	--	Alexa Fluor 488 ATTO 488	BB515 Cy2	CFSE DyLight 488	eYFP Fluorescein	FITC NL 493	
		710 / 50	--	BB700 PerCP-Vio700	PE-Alexa 647	PE-Alexa 700	PerCP-Cy5.5	PerCP-eFluor 710	
		585 / 15	--	PE					
LASER	561	610 / 20	--	Alexa Fluor 610 PE-CF594	CF594 PE-Dazzle 594	PE-Texas Red PE-eFluor 610	DyLight 594 PE-Vio615	ECD	
		670 / 30	--	PE-Cy5					
		710 / 50	--	PE-Cy5.5					
		780 / 60	--	PE-Cy7	PE-Vio770				
		670 / 14	--	APC	Alexa Fluor 647	Cy5	DyLight 633	NL 637	
LASER	640	730 / 45	--	Alexa Fluor 700	APC-R700				
				APC-Alexa 750	APC-CF770	APC-Cy7	APC-Vio770	eFluor 780 Fix Viability	
		780 / 60	--	Live/Dead Fix Near IR	APC-eFluor 780	APC-Fire 750	APC-H7		


# BD FACSAria II Cell Sorter - Training


- Sorting is a high skill level, so staff runs most sorts, but you must be an informed user!
- Know basic flow cytometry theory to make gates
- Watch BD Training: [Online Sorting Module](#)
- Watch OpenFlow videos: [Introduction to Cell Sorting Part I](#) [Introduction to Cell Sorting Part II](#)
- Read: [Reference Guide Cell Sorting Sample Preparation](#)
- Schedule meeting with FCCSF staff to discuss sort buffers, sample preparation, nozzle size, collection devices, sort mode, controls needed, etc.

# MACSQUANT TYTO CELL SORTER



- Cartridge based – sterility of sample can be maintained during sort
- Low pressure
- High speed
- Easy to use
- [Tyto Training Document](#)
- [Video Overview](#)

	<b>Violet 405 nm</b>	450/50 nm	V1	<b>VioBlue®</b> <b>Viobility™ 405/452</b> <b>Fixable Dye</b> <b>DAPI</b> <b>Hoechst 33342</b>	Alexa Fluor® 405 BD™ Horizon™ V450 BV421™ Calcein Violet 450 AM Cascade Blue® CFP	eBFP eFluor® 450 Hoechst Dyes Pacific Blue™ Vybrant® DyeCycle™ Violet Zombie Violet™
		525/50 nm	V2	<b>VioGreen™</b> <b>Viobility™ 405/520</b> <b>Fixable Dye</b>	Alexa Fluor® 430 AmCyan BD™ Horizon™ V500 BV510™ Cascade Yellow™	Krome Orange™ Pacific Orange™ Qdot® 525 Zombie Aqua™

	<b>Blue 488 nm</b>	525/50 nm	B1	<b>FITC</b> <b>VioBright™ FITC</b> <b>Vio® 515</b> <b>VioBright™ 515</b> <b>Viobility™ 488/520</b> <b>Fixable Dye</b>	Alexa Fluor® 488 Calcein AM DyLight® 488 CFSE GFP	SYTOX® Green Vybrant® DyeCycle™ Green YFP Zombie Green™
		585/40 nm	B2	<b>PE</b>	Cy™3	Vybrant® DyeCycle™ Orange
		655–730 nm	B3	<b>PerCP</b> <b>PerCP-Vio® 700</b> <b>PE-Vio® 615</b> <b>Propidium Iodide</b> <b>7-AAD</b>	PerCP-Cy™5.5 PE-Cy™5.5 PE-Cy™5 ECD PE-Texas Red®	BD™ Horizon™ PE-CF594 PE-eFluor® 610 PE-Alexa Fluor® 610 PE/Dazzle™ 594 PerCP-eFluor® 710
		750 nm LP	B4	<b>PE-Vio® 770</b>	PE-Alexa Fluor® 750	PE-Cy™7

	<b>Red 638 nm</b>	655–730 nm	R1	<b>APC</b> <b>Vio 667®</b> <b>VioBright™ 667</b>	Alexa Fluor® 647 Alexa Fluor® 700 APC-Alexa Fluor® 700	Cy™5 DRAQ5™ eFluor® 660
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## TYTO CONFIGURATION

- 3 lasers: 405, 488 and 638
- 8 fluorescent detectors plus backscatter blue, backscatter violet and backscatter red

# AGILENT SEAHORSE XFe96-TRAINING

- Real time, label-free cell metabolic analysis
- How it works: [Energy Pathways, Measuring Cell Metabolism and Drug Port Injection](#)
- [Integrated Imaging and Normalization System for more robust data](#)
- Training: [Seahorse training on Agilent website](#)
- Contact Seahorse Application Scientist for help with your specific assay [jay.dunn@agilent.com](mailto:jay.dunn@agilent.com)
- Schedule in iLab



# DATA ANALYSIS SOFTWARE

- 3 FlowJo dongles are available for checkout
- FlowJo annual Portal license may be purchased through the FCCSF for reduced price, usually less than \$350
- [FlowJo Recorded Webinars](#)
- Two computer analysis stations are available for use with FlowJo and SpectroFlow installed, located in MREB2 3226

# BD LSR FORTESSA X-20 – OTHER RESOURCES

- [Open Flow Cytometry YouTube channel](#)
- [Aja Reiger's YouTube channel](#)
- [flowMSKCC YouTube channel](#)
- [FlowJo Recorded Webinars](#)
- [FlowTex](#)

# GENERAL FLOW CYTOMETRY RESOURCES

- Introduction to Flow Cytometry by Melbourne Cytometry Platform -  
[https://biomedicalsciences.unimelb.edu.au/\\_data/assets/pdf\\_file/0012/3549837/MCP-introduction-to-flow-cytometry-final.pdf](https://biomedicalsciences.unimelb.edu.au/_data/assets/pdf_file/0012/3549837/MCP-introduction-to-flow-cytometry-final.pdf)
- Guidelines for the use of flow cytometry and cell sorting in immunological studies (third edition), Cossarizza et. Al, 2021-exhaustive references with methods included <https://pubmed.ncbi.nlm.nih.gov/34910301/>
- Flow Cytometry Today \_Springer 2022